THE DIFFERENTIAL EFFECTS OF BUPRENORPHINE AND METHADONE ON ADOLESCENT MICE

An Undergraduate Research Scholars Thesis

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ABSTRACT

The Differential Effects of Buprenorphine and Methadone on Adolescent Mice (May 2013)

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This study focuses on understanding the consequences of administering opioid maintenance drugs, such as buprenorphine and methadone, on the adolescent brain. This is because that there are growing needs to treat opioid dependence and addiction in adolescents and there is a lack of studies that aim at explaining the effects of opioid maintenance drugs on the still-developing adolescent brain. The goal of this study is to enable physicians to conduct a science-based risk assessment on the proper use of these treatments for that age group. A recent study conducted in our lab has indicated potential risks in chronic exposure to certain opioids, specifically, morphine during adolescence. Morphine exposure was observed to change the signaling of D2/D3 dopamine receptors in adolescent mice. The D2/D3 dopamine receptor may mediate long-term mental changes in adolescents, specifically changes linked to mood and psychotic disorders. Thus, this study examined whether buprenorphine and methadone alter the responses of the D2/D3 dopamine receptors differently in an adolescent population. Adolescent mice were orally administered buprenorphine, methadone, or saline once daily for 6 days. Two hours or three days later, the mice were tested for their locomotor response to quinpirole, a D2/D3 dopamine receptor agonist. Buprenorphine-treated adolescent mice showed a similar response to that of the

drug-naïve (saline-injected) group in their response to quinpirole. In contrast, an enhanced response was observed in methadone-treated adolescent animals. This effect was significantly higher two hours following the final dose of methadone, as compared to three days afterwards. As shown in this study, methadone exposure greatly disturbs the D2/D3 receptor's signaling. This indicates that care should be taken when administering methadone to adolescents for addiction therapies as well as pain management. In contrast to methadone, buprenorphine appears to disrupt the D2/D3 dopamine receptor signaling in adolescents less. Therefore, this study may confirm that exposure to different opioids carries different risks, specifically in an adolescent population.

CHAPTER I

INTRODUCTION

The abuse of drugs, specifically opioids, is becoming more prevalent in adolescent populations. Next to marijuana, opioids are the most common form of illicit drug use in the United States[1]. Furthermore, the nonmedical use of pain relievers has shown to be more prevalent than the nonmedical use of stimulants, sedatives, and tranquilizers[2]. These pain relievers are almost invariably an opioid. Because of this, understanding the mechanism of action as well as developing ways to combat the risks associated with the use of opioids is becoming an important area for study, specifically when approaching adolescent populations.

Currently, there is a small amount of research on how opioids differentially affect adults versus adolescents. What our recent research has attempted to highlight is the fact that there are differences in how opioids affect adolescents in comparison to adults and the fact that there are varying effects between different opioids on these populations. When considering neural activity, there may be many factors that contribute to this fact, but the continual development of the adolescent brain is one of the most important[3]. In any case, it is appropriate to suggest that there are differences in how opioids act in each population, and that different opioids carry differing effects due to their varying molecular profiles[4]. Furthermore, how we go about treating drug-dependence in these two populations should be carefully approached.

In support of these hypotheses, we recently observed that morphine differentially alters the responses of the D2/D3 dopamine receptors in adults and adolescents.

Following repeated exposure to morphine, we found that adolescents experienced an intensified response upon exposure to a D2/D3 dopamine receptor agonist, a compound that increases the activity of this receptor system[5]. Morphine altered the response of the D2/D3 dopamine receptors in adults, but to a significantly less extent than the response observed in adolescents.

Although this effect might possibly be seen in other receptor-signaling pathways, our focus on the D2/D3 receptor is anchored in its implications in mental health and mediation of mood and psychotic disorders such as schizophrenia, depression, and bipolar disorder[6]. Given there are differences in the actions of different drugs on this system, as well as differences amongst adults and adolescents in terms of the actions of these drugs, value can be found in a study that can determine the side-effects/risks associated with opioids in each population on this D2/D3 receptor-signaling system. Especially when considering how important the D2/D3 receptor-signaling system is to mental status.

Buprenorphine and methadone are primarily known for their use as maintenance treatments in human opioid addiction therapy, mainly for adults. However, with restriction and limitation, these drugs are also used for adolescents. In addition, buprenorphine and methadone are also used for pain management in both populations and methadone is becoming a commonly abused opioid by adolescents[3,7]. Both drugs have been shown to be effective in treating addiction. However, many warnings have been issued in association with the use of them. Surprisingly, there are a very small amount of studies that focus on the specific dangers associated with the two drugs, especially for adolescents. Given the importance of D2/D3 dopamine receptors to mental health, this study has focused on the differing effects of methadone and buprenorphine on

the D2/D3 receptor-signaling pathway. Moreover, because there is a gap in the literature on the effects of opioids on adolescent populations, we chose to focus our research on this population. Lastly, to increase the clinical relevancy of this research, the mice were administered the drugs via gavage injection (oral). This is because humans are most commonly administered pain/maintenance treatments via oral administration (pills). The knowledge acquired in these studies will hopefully assist psychiatrists and other physicians in developing a safer treatment regimen for adolescents receiving both addiction maintenance therapy and pain management therapy.

In this study we examined the effects of buprenorphine and methadone on the D2/D3 dopamine receptor-signaling in adolescent mice. We used similar methodology to those used in our previous study with morphine. In the first experiment, adolescent mice were administered once daily for six days with one of three different doses of methadone (25, 50, or 100 mg/kg) or buprenorphine (.1, .2, .4 mg/kg) or a saline solution. Three days later the mice were examined for their locomotor response to quinpirole, a D2/D3 receptor agonist. In the second experiment, mice were administered similarly with either 50 mg/kg methadone, 0.2 mg/kg buprenorphine or saline and their response to quinpirole was tested 2 hours after the last injection. This experiment was done in order to determine whether the effects of the opioids on D2/D3 dopamine receptor-signaling are due to the withdrawal effects or the actual drugs themselves. In the third experiment, we examined the blood plasma levels of methadone and buprenorphine after six days of administration to confirm that the drug levels are within the relevant range when compared to humans receiving therapeutic doses of each drug.

CHAPTER II

MATERIALS AND METHODS

Animals

All procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Adolescent male C57BL/6 mice were purchased from Harlan Labs in Houston, TX. Mice were housed in groups of 4 and supplied with rodent food and water ad lib. The vivarium was temperature controlled (21 ± °C) with a 12 h/12 h light/dark cycle for approximately one week prior to treatment, allowing for acclimation of the mice to the environment. Mice were determined to be in the adolescent phase based on studies by Spear and colleagues, which described the three developmental stages for rodents from weaning to adulthood. In accordance with these studies, mice were purchased at postnatal day 22 (PND 22). They were allowed to acclimate to the vivarium until the age of PND 28. Injections of methadone, buprenorphine, or saline began on PND 28, and behavioral testing was performed on PND 33 or 36. Accordingly, mice were injected during late prepubescent period and were tested during their mid-adolescent period.

Treatment regimen

Adolescent mice (n=13-31 per experimental group) were administered buprenorphine (0.1, 0.2, or 0.4 mg/kg), methadone (25, 50, or 100 mg/kg) or saline once daily for six days via gavage injection. Methadone and Buprenorphine were purchased from Sigma-Aldrich Chemicals (St Louis, MO). Doses were given to represent plasma levels that are representative of a human

therapeutic dose[8,9,10]. The dose was selected in accordance with existing literature on the pharmacokinetics of the selected drugs in mice[11].

Locomotion testing

8 activity chambers were each equipped with optical beam activity monitors (Model RXYZCM-16; Accusan Instruments, Columbus, OH, USA) and were used to assess the activity of the mice. The chambers consisted of a 40 X 40 X 30.5 cm acrylic cage with the optical beam monitors positioned around this cage. This system of chambers was set up in a sound-proof room equipped with a 40 dB white noise producer operating at all times when mice were present in the room. When mice are present in the chambers, a multiplexor-analyzer tracks the mice by monitoring the interruption of beams from the optical beam monitors. The position of the mice in the acrylic chambers is updated every 10 ms using a real-time computerized conversion system. Activity measurements are obtained from the computerized sorting of the data.

Two-hour withdrawal procedure

Following the last injection of buprenorphine, methadone, or saline solution, the mice were left in the vivarium for 1 hour. Immediately following, the mice were moved to the activity room for a 30 minute habituation period. The mice were then placed, one per chamber, into the center of the activity chambers, and a 30 minute baseline period was recorded. Once the baseline period was complete, the mice were injected via IP with either quinpirole (10 mg/kg) or a vehicle solution and were placed back into their respective chambers for a 120 minute locomotor session. Immediately after this session, mice were placed back into their cage and moved out of the activity room. Using pentobarbital (100 mg/kg) via IP injection, the mice were euthanized.

After euthanasia, the brains were taken and frozen by placing them in a plastic container and setting the container into a mixture of dry ice and acetone. Once all of the brains were removed and completely frozen, they were preserved at -80°C.

Three day withdrawal procedure

Following the last administration of buprenorphine, methadone, or saline, mice were left in the vivarium for 3 days. On the 3rd day, the mice were moved into the activity room for a 30 minute habituation period. All procedures from this point are identical to the two hour withdrawal procedure described above.

Plasma levels of buprenorphine and methadone

As described above, mice (n=8-9 per group) were administered 0.2 mg/kg buprenorphine or 50 mg/kg methadone for six days. Two, 6, or 24 hours following final treatment of drug, mice were anesthetized using pentobarbital (100 mg/kg) via intraperitoneal injection. Immediately following, blood was collected via intra-cardiac puncture with syringes lined with heparin. Plasma was immediately separated via centrifugation (15 min, 1000g, 4°C) and stored at -80°C. Buprenorphine and methadone levels from the acquired plasma was determined using an ELISA Kit purchased from Neogen Corporation (St. Joseph, MI).

Data analysis

Total distance traveled (cm) scores for each mouse during the 120 minute post-vehicle or post-quinpirole period were normalized to the total distance traveled (cm) during the 30 minute baseline activity using the formula: [total distance traveled post-vehicle or post-quinpirole/total distance traveled (cm) during baseline] X 100. Data for between-subjects factor of treatment was analyzed for the normalized total distance traveled scores (% from baseline) during the 120 minutes post-vehicle or post-quinpirole using the Univariate Analysis of Variance (SPSS Statistics 18, Somers, NY). Additional temporal analysis of a factorial consisting of between-group factors of treatment (buprenorphine, methadone, or saline) and within-group factor of time (1-120 minutes post-injection period summed in 5 minute intervals) was also computed. For this analysis, for each mouse the score of the last 5-minute interval prior to the vehicle or quinpirole injections (baseline) was used to normalize the data. Post-hoc contrasts between each treatment group were computed using Bonferroni post-hoc procedure. Differences with p-values of less than 0.05 were deemed statistically significant. Results are shown as mean +/- SEM.

CHAPTER III

RESULTS

Experiment I: Three-day withdrawal

Total distance traveled post-vehicle

When mice were administered vehicle immediately prior to the 120 minute test period, a Univariate Analysis of Variance revealed no significant differences in locomotor activity levels between drug-naïve mice and mice administered the various doses of buprenorphine and methadone ($F(6, 81)=0.73$, $p>0.05$, n.s.) (Shown in Figure 1a).

Total distance traveled post-quinpirole

When mice were administered quinpirole immediately prior to the 120 minute locomotor test period, a Univariate Analysis of Variance revealed significant differences in activity levels between mice treated with the various drugs ($F(6, 117)=4.38$, $p<0.0001$). Post hoc comparison revealed no differences in quinpirole-induced suppression of activity between the saline-injected mice and mice treated with 0.1, 0.2, and 0.4 mg/kg buprenorphine. Significant differences were revealed between saline-injected mice (drug-naïve) and mice treated with 25, 50, and 100 mg/kg methadone ($p<0.05$). The mice were observed to have less of a decrease in activity when administered quinpirole in contrast with the saline-injected (drug-naïve) mice.

Temporal analysis post-vehicle

Temporal analyses were computed by taking 5 minute intervals of the 120 minutes post-vehicle injection and calculating distanced traveled scores for each interval. Figure 2a presents the results for the buprenorphine-treated animals. Two-way repeated-measure ANOVA revealed a main effect of time ($F(23, 1058)=7.13$, $p<0.0001$), but no significant main effect of treatment ($F(3, 46)=0.14$, $p>0.05$, n.s.) and no significant interaction between treatment and time ($F(69, 1058)=1.06$, $p>0.05$, n.s.). Activity levels declined as the test period proceeded. However, there were no significant differences in activity levels between drug-naïve mice and mice administered 0.1, 0.2, or 0.4 mg/kg buprenorphine. Figure 2b presents the results for the methadone-treated animals. Two-way repeated-measure ANOVA revealed a main effect of time ($F(23, 1058)=5.78$, $p<0.0001$), but no significant main effect of treatment ($F(3, 46)=0.12$, $p>0.05$, n.s.), and no significant interaction between treatment and time ($F(69, 1058)=1.08$, $p>0.05$, n.s.). Activity levels were observed to decrease as the test period proceeded. However, as with buprenorphine, no differences in their activity levels were observed between drug-naïve mice and mice administered 25, 50, or 100 mg/kg methadone.

Temporal analysis post-quinpirole

Figure 2c presents the temporal analysis following quinpirole treatment for the buprenorphine treated animals. Two-way repeated-measure ANOVA revealed a main effect of time ($F(23, 1633)=13.37$, $p<0.0001$), but no significant main effect of treatment ($F(3, 71)=1.68$, $p>0.05$, n.s.), and no significant interaction between treatment and time ($F(69, 1633)=0.87$, $p>0.05$, n.s.). Post hoc comparison revealed no differences in quinpirole-

induced attenuation of activity level between drug-naïve mice (i.e. saline-injected mice) and mice treated with the various doses of buprenorphine. Similar to the drug-naïve animals, the buprenorphine treated animals showed a steady increase in locomotor activity starting at 10 minutes following buprenorphine administration, with the locomotor response peaking at 25 minutes. From the peak at 25 minutes, a steady decline in locomotor activity was observed followed by a very gradual increase in locomotor activity for the remainder of the session. At 120 minutes, the 0.4 mg/kg buprenorphine-treated animals showed the greatest increase in locomotor activity while the 0.2 mg/kg buprenorphine-treated animals showed the least increase in locomotor activity. However, no significant differences from saline-injected control animals were observed at any of the time points.

Figure 2d presents the results for the temporal analysis following quinpirole treatment for the methadone-treated animals. Two-way repeated-measure ANOVA revealed a main effect of time ($F(23, 1702)=21.37$, $p<0.0001$), a main effect of treatment ($F(3, 74)=3.41$, $p<0.05$), and a significant interaction between treatment and time ($F(69, 1702)=2.61$, $p<0.0001$). In contrast to buprenorphine, significant differences in the locomotor response to quinpirole between drug-naïve mice and mice treated with the various doses of methadone were observed in the 5 minute interval periods during the 120 minutes test period following administration of quinpirole ($p<0.05$). Specifically, each of the methadone-treated animals showed a sharp increase in locomotor activity starting 20 minutes following quinpirole administration, with the locomotor response peaking at 30 minutes. Immediately

following the peak of the locomotor response, a sharp decline was observed until 40 minutes following quinpirole administration. From 40 minutes following quinpirole administration until the remainder of the session, we observed a steady increase in locomotor activity for the methadone-treated animals. At 120 minutes, the 100 mg/kg methadone-treated animals showed the greatest increase in locomotor activity, while the 25 mg/kg methadone treated animals showed the least increase in locomotor activity.

Experiment II: Two-hour withdrawal

Total distance traveled post-vehicle

Figure 3a presents the results for the total distance traveled scores (% from baseline) during the 120 minutes following treatment with vehicle. Univariate Analysis of Variance revealed no significant differences in activity levels between drug-naïve mice and mice administered with 0.2 mg/kg buprenorphine or 50 mg/kg methadone ($F(2, 37)=0.154$, $p>0.05$, n.s.).

Total distance traveled post-quinpirole

Fig 3b presents the results for the total distance traveled (% from baseline) during the 120 minutes following treatment with quinpirole. Univariate Analysis of Variance revealed significant differences in the locomotor response to quinpirole between animals treated with the various drugs ($F(2, 28)=18.53$, $p<0.0001$). Post hoc comparison revealed no significant differences in quinpirole-induced suppression of activity level between drug-naïve mice (i.e. saline-injected mice) and mice treated with 0.2 mg/kg buprenorphine. In

comparison, a significant difference was observed between drug-naïve mice and mice treated with 50 mg/kg methadone ($p<0.05$). Quinpirole significantly increased the activity levels of methadone-treated animals in comparison to the locomotor activity of the drug-naïve animals.

Temporal analysis post-vehicle

Temporal analysis was computed using the distance-traveled scores during 5 minute intervals of the 120 minutes post-vehicle injection as presented in figure 4a. Two-way repeated-measure ANOVA revealed a main effect of time ($F(23, 851)=13.82$, $p<0.0001$), but no significant main effect of treatment ($F(2, 37)=0.68$, $p>0.05$, n.s.) and no significant interaction between treatment and time ($F(46, 851)=1.22$, $p>0.05$, n.s.). Reduced activity was observed for all treatment groups as the experiment proceeded. However, no differences in their activity levels were observed between drug-naïve mice and mice administered with 0.2 mg/kg buprenorphine or 50 mg/kg methadone.

Temporal analysis post-quinpirole

Figure 4b presents the temporal analysis following treatment with quinpirole. Two-way repeated-measure ANOVA revealed a main effect of time ($F(23, 644)=13.36$, $p<0.0001$), a main effect of treatment ($F(2, 28)= 23.06$, $p<0.0001$), and a significant interaction between treatment and time ($F(46, 644)=11.95$, $p<0.0001$). Post hoc comparison revealed no differences in quinpirole-induced suppression of activity level between drug-naïve mice (i.e. saline-injected mice) and mice treated with 0.2 mg/kg buprenorphine. In comparison, a significant difference was observed between drug-naïve mice and mice treated with 50

mg/kg methadone ($p<0.05$). Starting at 10 minutes following quinpirole treatment until the end of the testing period, significant differences were observed between drug-naïve and methadone-treated animals in their locomotor response to quinpirole throughout each of the 5 minute interval periods. Specifically, the methadone-treated animals showed a sharp increase in locomotor activity 10 minutes after quinpirole administration, followed by a steady increase in locomotor activity for the remaining 100 minutes. At 120 minutes following quinpirole administration, the methadone-treated animals reached an activity level approximately 350% of baseline, while the buprenorphine and saline-treated animals reached an activity level slightly above baseline.

Plasma levels of buprenorphine and methadone

Following six days of oral administration of 0.2 mg/kg buprenorphine, a peak plasma concentration of 7.6 ± 0.8 ng/ml was observed 2 hours after the last dose of buprenorphine, as presented in figure 5a. Following six days of oral administration of 50 mg/kg methadone a peak plasma concentration of 133 ± 28 ng/ml was observed 6 hours after the last dose of methadone, as presented in figure 5b.

CHAPTER IV

CONCLUSIONS

This study provides evidence that buprenorphine and methadone affect the locomotor response to a D2/D3 dopamine receptor agonist in differing ways. Quinpirole, a D2/D3 receptor agonist, reduced locomotor activity in drug-naïve (saline-injected) adolescent mice, lasting for the entire 120 minute session. This response is consistent with literature that provides evidence that the suppressive effect of quinpirole on motor activity is pronounced in mice[12]. Our results show that buprenorphine-treated animals showed a response to quinpirole similar to the response seen in control drug-naïve animals. That is, no effects on the response to quinpirole were seen two hours or three days following final treatment of buprenorphine. In contrast, animals treated with methadone showed an enhanced locomotor response after an initial suppression when administered quinpirole. This response was much greater in the animals receiving quinpirole two hours following final treatment of methadone, as opposed to three days later. This suggests that methadone itself and not the withdrawal effects, is modifying the D2/D3 receptor-signaling system.

The ability for quinpirole to reduce locomotor activity has been suggested to be due to the attenuation of dopamine release[13]. This effect at the presynaptic neuron is mediated by its activity specifically at the short isoform of the D2 dopamine receptor[14,15]. In contrast, the motor activating effect of quinpirole is thought to be mediated by D2L/D3 postsynaptic receptors[16,17]. In our experiments, quinpirole-induced suppression of the locomotor response was still observed

in the methadone-treated animals in the first 10 minutes. This suggests that the presynaptic D2 signaling or release of dopamine is not altered and the main effect is present at the postsynaptic D2L/D3 dopamine receptor signaling.

Hyper-sensitivity to D2/D3, but not D1, dopamine receptor agonists was observed in adult rats undergoing morphine withdrawal[18,19,20]. Recently, our group has observed that this supersenstivity of the D2/D3L postsynaptic receptor signaling is greatly increased in adolescents as compared to adults following administration of morphine[21]. This study extends those results and demonstrates that other opioids, such as methadone, have disturbing effects on the D2L/D3 postsynaptic receptor signaling. In addition, these studies suggest that different exposure to different opioids (morphine and methadone vs. buprenorphine) carries different risks in altering the dopaminergic system of adolescent populations. Furthermore, this study suggests that the enhanced effect in adolescents is probably a direct effect of opioid exposure, rather than the effect of withdrawal, due to the fact that there was more of an effect observed two hours following the last methadone administration when compared to the three day withdrawal animals.

To confirm that the doses of buprenorphine and methadone used in this study are within the range used clinically in human , the plasma levels were recorded in the mice 2, 6, and 24 hours following the last administration of 0.2 mg/kg buprenorphine and 50 mg/kg methadone. The dosage of sublingual buprenorphine for adults recommended by the American Psychiatric Association for maintenance treatment of opioid-dependent individuals, ranges from 2 to 32 mg, daily to 3 times a week. A similar range of 4 to16 mg

tablets are reported for the treatment of adolescents[22]. Buprenorphine plasma levels in male patients maintained on 16 mg sublingual tablets was recorded to be roughly 40 ng×h/ml over a 24 hour period, reaching a maximum plasma concentration of about 5 ng/ml 1.2 hours after administration[23]. A wide range of oral methadone doses are used in patients, with the recommendations generally ranging from 20-100 mg daily. A similar range was also used for clinical studies in adolescents, with an average maintenance dose of approximately 50 mg[22]. Therapeutic methadone plasma levels range between 100-1000 ng/ml, with studies also showing that plasma levels exceeding 200 ng/ml are usually required for effectiveness. A plasma range of 400-500 ng/ml is recommended for optimal therapeutic efficiency. Based on these studies, the plasma levels of the methadone-treated animals were determined to be lower than that of plasma levels in a human therapeutic dose. Therefore, the enhanced locomotor response observed in the methadone-treated animals was not a result of an overly high dose of methadone; rather these effects were seen at suboptimal doses. This suggests that the D2/D3 receptor signaling system is altered even at low plasma levels of methadone. The plasma levels following administration of 0.2 mg/kg buprenorphine, however, were shown to be higher than what is expected from a human therapeutic dose. This suggests that the negligible effect seen at the D2/D3 postsynaptic receptor is not due to a suboptimal dose of buprenorphine.

Disruption of the dopamine receptor signaling may have long term effects, specifically involving the use of opioids. The D2 dopamine receptors are important in the reinforcing properties associated with morphine[24], and also in mediating drug-seeking behaviors in mice[25] and rats[26] undergoing morphine withdrawal. The D3 dopamine receptor has been

demonstrated to play a role in opioid sensitization[27,28] and reward[28,29,30]. In addition to affecting opioid abuse, there are many other possible implications involved with disrupting the D2/D3 dopamine receptors. It is suggested D2 receptor genes may play a role in the abuse of various illicit drugs and alcoholism[31,32,33]. The pathophysiology of affective and psychotic disorders have also shown to potentially be mediated by the D2/D3 receptor system[34,35,36,37].

As demonstrated in this study, mice treated with methadone exhibited supersensitivity to a D2/D3 dopamine receptor agonist, but this effect was not present in mice treated with buprenorphine. Therefore, it is suggested that adolescents exposed to methadone may exhibit markedly robust disturbances of the D2/D3 receptor signaling. Due to the fact that the dopaminergic system is heavily involved in the pathophysiology of many mental illnesses, this study suggests that extra care must be taken when contemplating treatment of adolescents with this drug in an opioid dependence application as well as a pain management application. In contrast to methadone, buprenorphine appears to disrupt the D2/D3 dopamine receptor signaling in adolescents less. Thus, these results also suggest that different opioids carry different risks in altering the highly sensitive neurochemistry of the adolescent brain, especially at the D2/D3 receptor level. Although this study indicates that buprenorphine might be safer to use than other opioids for treatment in adolescents, cross-sensitization may develop between various opioids. Therefore, it is possible that the effects of buprenorphine may differ in opioid-dependent adolescents as compared to drug-naïve adolescents. Thus, further studies need to be conducted to examine the effect of buprenorphine in adolescents that are previously exposed to other opioids (either in the

course of treatment or recreationally). It is also possible that the level of cross-sensitization will differ between buprenorphine and various opioids.

REFERENCES

1. SAMHSA (2009): *The NSDUH report: trends in nonmedical use of prescription pain relievers: 2002 to 2007*. Rockville, MD: Substance Abuse and Mental Health Services Administration, Office of Applied Studies
2. Young, April M., Natalie Glover, and Jennifer R. Havens (2012): Nonmedical Use of Prescription Medications Among Adolescents in the United States: A Systematic Review. *Journal of Adolescent Health* 51.1: 6-17
3. Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE (2011): Postnatal brain development: structural imaging of dynamic neurodevelopmental processes. *Prog Brain Res* 189:77-92.
4. Arttamangkul S, Quillinan N, Low MJ, von Zastrow M, Pintar J, Williams JT (2008): Differential activation and trafficking of micro-opioid receptors in brain slices. *Molecular Pharmacology* 74:972-979.
5. Hofford RS, Wellman PJ, Eitan S (2012): Morphine alters the locomotor responses to a D2/D3 dopamine receptor agonist differentially in adolescent and adult mice. *Journal of Psychopharmacology* In press.
6. Kehne, John H., Terrance H. Andree, and Julia N. Heinrich (2008): D2 Receptor Partial Agonists: Treatment of CNS Disorders of Dopamine Function. *Current Topics in Medicinal Chemistry* 8.12: 1068-088
7. Kleber HD, Weiss RD (2006): Practice Guideline for the Treatment of Patients With Substance Use Disorders, Second Edition. *American Psychiatric Association*.

8. Bell J, Mutch C (2006): Treatment retention in adolescent patients treated with methadone or buprenorphine for opioid dependence: a file review. *Drug Alcohol Rev* 25:167-171
9. Institution (2003) *Methadone Dosing & Safety in the Treatment of Opioid Addiction*. Mundelein, IL: Institution.
10. Jagsch R, Gombas W, Schindler SD, Eder H, Moody, DE, Fischer G (2005): Opioid plasma concentrations in methadone and buprenorphine-maintained patients. *Addiction Biology* 10:365-371.
11. Kalliokoski O, Jacobsen KR, Hau J, Abelson KS (2011): Serum concentrations of buprenorphine after oral and parenteral administration in male mice. *Vet J* 187:251-254.
12. Halberda JP, Middaugh LD, Gard BE, Jackson BP (1997) DAD1- and DAD2-like agonist effects on motor activity of C57 mice: differences compared to rats. *Synapse* **26**:81-92.
13. Starke K, Gothert M, Kilbinger H (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol Rev* **69**:864-989.
14. Mogenson GJ, Wu M (1991) Effects of administration of dopamine D2 agonist quinpirole on exploratory locomotion. *Brain Res* **551**:216-20.
15. Usiello A, Baik J-H, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, Piazza PV, Borrelli E (2000) Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* **408**:199-203.
16. Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M (2004) The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined

behavioural and neurochemical analysis with novel, selective antagonists in rats. *Psychopharmacology (Berl.)* **174**:341-357.

17. Marsteller DA, Gerald CPG, Kong R, Cajina M, Craig DA, Swanson CJ (2009) The MCH1 receptor antagonist SNAP 94847 induces sensitivity to dopamine D2/D3 receptor agonists in rats and mice. *Eur J Pharmacol* **602**:66-72.
18. Lee JM, DeLeon-Jones F, Fields JZ, Ritzmann RF (1987) Cyclo (Leu-Gly) attenuates the striatal dopaminergic supersensitivity induced by chronic morphine. *Alcohol Drug Res.* **7**:1-10.
19. Piepponen TP, Katajamäki J, Kivastik T, Zharkovsky A, Ahtee L (1996) Behavioural and neurochemical sensitization of morphine-withdrawn rats to quinpirole. *Pharmacol Biochem Behav* **54**:787-792.
20. Druhan JP, Walters CL, Aston-Jones G (2000) Behavioral activation induced by D(2)-like receptor stimulation during opiate withdrawal. *J Pharmacol Exp Ther* **294**:531-8.
21. Hofford RS, Wellman PJ, Eitan S (2012) Morphine alters the locomotor responses to a D2/D3 dopamine receptor agonist differentially in adolescent and adult mice. *J Psychopharm* In press.
22. Bell J, Mutch C (2006) Treatment retention in adolescent patients treated with methadone or buprenorphine for opioid dependence: a file review. *Drug Alcohol Rev* **25**:167-71.
23. Moody DE, Fang WB, Morrison J, McCance-Katz E (2011) Gender differences in pharmacokinetics of maintenance dosed buprenorphine. *Drug Alcohol Depend* **118**:479-83.

24. Elmer GI, Pieper JO, Rubinstein M, Low MJ, Grandy DK, Wise RA (2002) Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. *J Neurosci* **22**:RC224.
25. Dockstader CL, Rubinstein M, Grandy DK, Low MJ, van der Kooy D (2001) The D2 receptor is critical in mediating opiate motivation only in opiate-dependent and withdrawn mice. *Eur J Neurosci* **13**:995-1001.
26. De Vries TJ, Schoffelmeer AN, Binnekade R, Vanderschuren LJ (1999) Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. *Psychopharmacology (Berl)* **143**:254-60.
27. Cook CD, Beardsley PM (2003) The modulatory actions of dopamine D2/3 agonists and antagonists on the locomotor-activating effects of morphine and caffeine in mice. *Pharmacol Biochem Behav* **75**:363-71.
28. Narita M, Mizuo K, Mizoguchi H, Sakata M, Tseng LF, Suzuki T (2003) Molecular evidence for the functional role of dopamine D3 receptor in the morphine-induced rewarding effect and hyperlocomotion. *J Neurosci* **23**:1006-12.
29. Ashby CR, Jr., Paul M, Gardner EL, Heidbreder CA, Hagan JJ (2003) Acute administration of the selective D3 receptor antagonist SB-277011A blocks the acquisition and expression of the conditioned place preference response to heroin in male rats. *Synapse* **48**:154-6.
30. Frances H, Smirnova M, Leriche L, Sokoloff P (2004) Dopamine D3 receptor ligands modulate the acquisition of morphine-conditioned place preference. *Psychopharmacology (Berl)* **175**: 127-33.

31. Blum K, Noble EP, Sheridan PJ, Finley O, Montgomery A, Ritchie T, Ozkaragoz T, Fitch RJ, Sadlack F, Sheffield D, et al. (1991) Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. *Alcohol* **8**:409-16.
32. Esposito-Smythers C, Spirito A, Rizzo C, McGeary JE, Knopik VS (2009) Associations of the DRD2 TaqIA polymorphism with impulsivity and substance use: preliminary results from a clinical sample of adolescents. *Pharmacol Biochem Behav* **93**:306-12.
33. Nemoda Z, Szekely A, Sasvari-Szekely M (2011) Psychopathological aspects of dopaminergic gene polymorphisms in adolescence and young adulthood. *Neurosci Biobehav Rev*
34. Xu R, Hranilovic D, Fetsko LA, Bucan M, Wang Y (2002) Dopamine D2S and D2L receptors may differentially contribute to the actions of antipsychotic and psychotic agents in mice. *Mol Psychiatry* **7**:1075-82.
35. Svestka J (2005) Treatment of bipolar depression. *Neuro Endocrinol Lett* **26 Suppl 1**:27-47.
36. Gershon AA, Vishne T, Grunhaus L (2007) Dopamine D2-like receptors and the antidepressant response. *Biol Psychiatry* **61**:145-53.
37. Seeman P (2011) All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D2(high) receptors. *CNS Neurosci Ther.* **17**:118-32.

FIGURES

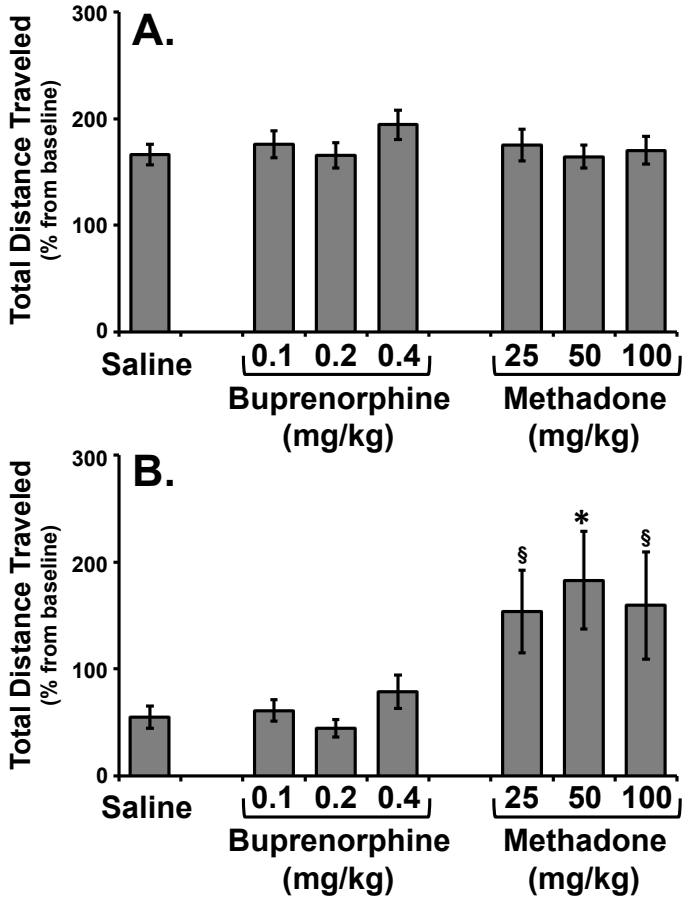

Figure 1 Mice were orally administered buprenorphine (0.1, 0.2 or 0.4 mg/kg) or methadone (25, 50, or 100 mg/kg) for 6 days. Three days after the final injection, their response to vehicle (**A**) or 10 mg/kg quinpirole (**B**) was tested. Results are presented as mean ± SEM of the total distance traveled scores (% from baseline) in the 120 minutes after administration of vehicle or quinpirole. (*) indicates a significant difference from saline-treated mice ($p<0.05$, Bonferroni); (§) indicates a significant difference from saline-treated mice ($p<0.05$, LSD).

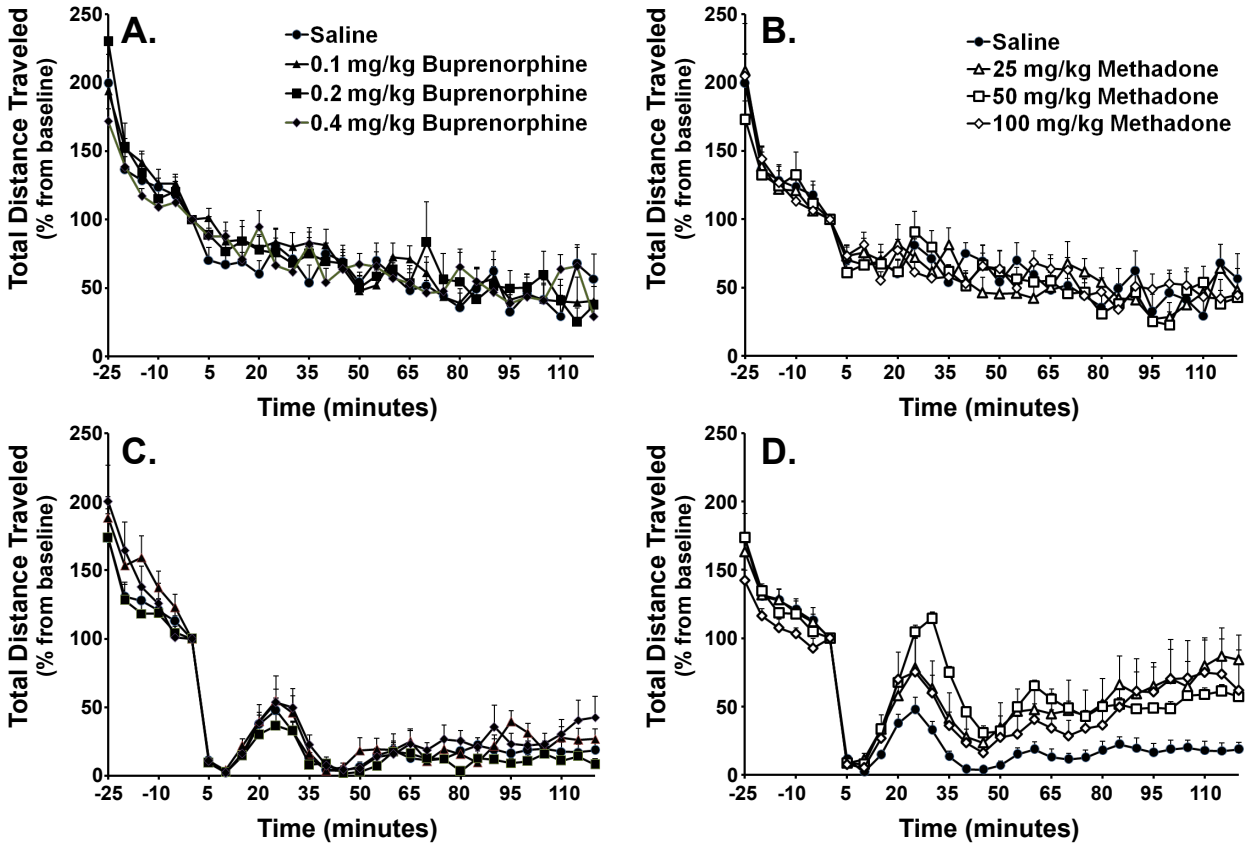

Figure 2 Mice were orally administered buprenorphine (0.1, 0.2 or 0.4 mg/kg; **A** and **C**) or methadone (25, 50, or 100 mg/kg; **B** and **D**) for 6 days. Three days following the last injection, their response to vehicle (**A** and **B**) or 10 mg/kg quinpirole (**C** and **D**) was tested. Results are presented as mean ± SEM of the total distance traveled scores (% from baseline) for each 5 minute interval during the 30 minute baseline and 120 minutes after administration of vehicle or quinpirole. Each time point represents the 5 minute interval immediately preceding that time. Quinpirole was administered at t=0. Significant differences in the locomotor response to quinpirole between drug-naïve mice and mice treated with the various doses of methadone were observed in multiple 5 minute interval periods during the 120 minutes post-quinpirole ($p<0.05$,

Bonferroni). However, these post hoc contrasts are not presented to maintain the simplicity of the figure.

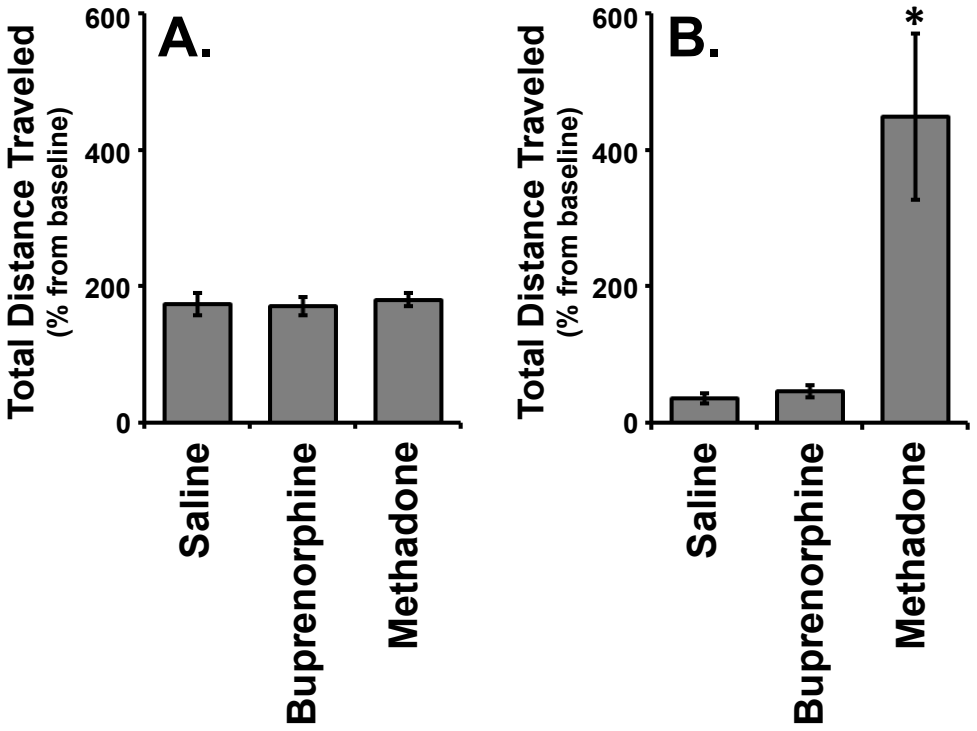

Figure 3 Mice were orally administered 0.2 mg/kg buprenorphine or 50 mg/kg methadone for 6 days. Two hours after the final dose, their response to vehicle (**A**) or 10 mg/kg quinpirole (**B**) was tested. Results are presented as mean ± SEM of the total distance traveled scores (% from baseline) in the 120 minutes after administration of vehicle or quinpirole. (*) indicates a significant difference from saline-treated mice ($p<0.05$, Bonferroni).

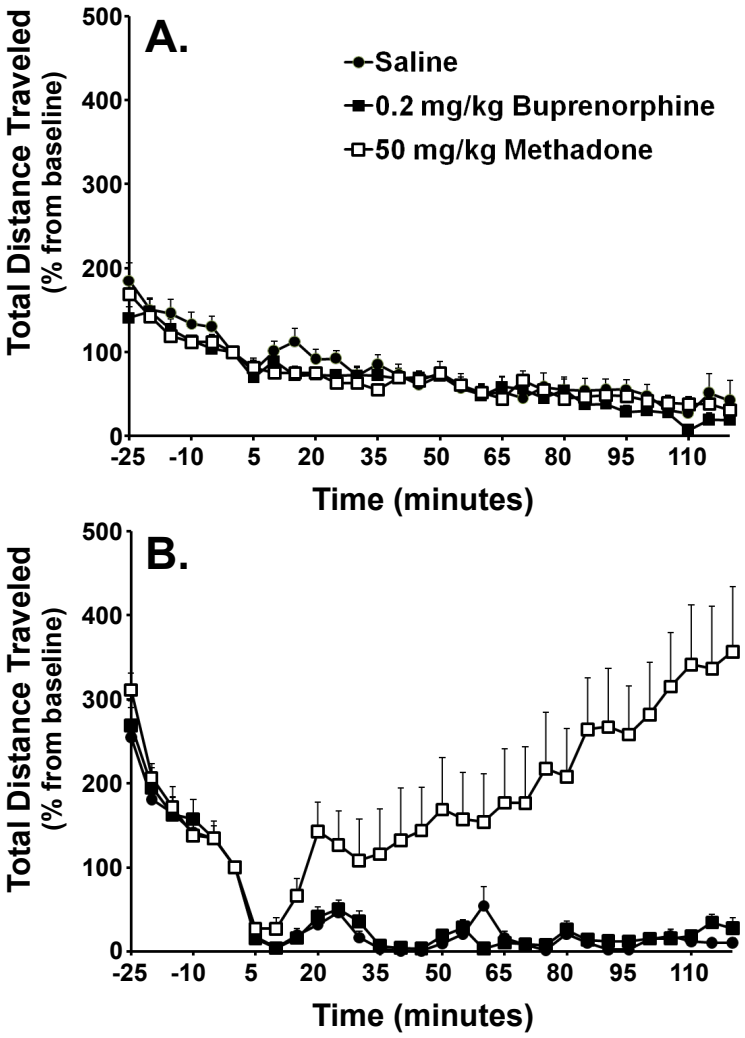

Figure 4 Mice were orally administered 0.2 mg/kg buprenorphine or 50 mg/kg methadone for 6 days. Two hours after the last dose, their response to vehicle (**A**) or 10 mg/kg quinpirole (**B**) was tested. Results are presented as mean ± SEM of the total distance traveled scores (% from baseline) for each 5 minute interval during the 30 minute baseline and 120 minutes after administration of vehicle or quinpirole. Each time point represents the 5 minute interval immediately preceding that time. Quinpirole was administered at t=0. In each 5 minute interval period from t=10 until the end of the testing period, significant

differences were observed between drug-naïve and methadone-treated animals in their locomotor response to quinpirole ($p<0.05$, Bonferroni).

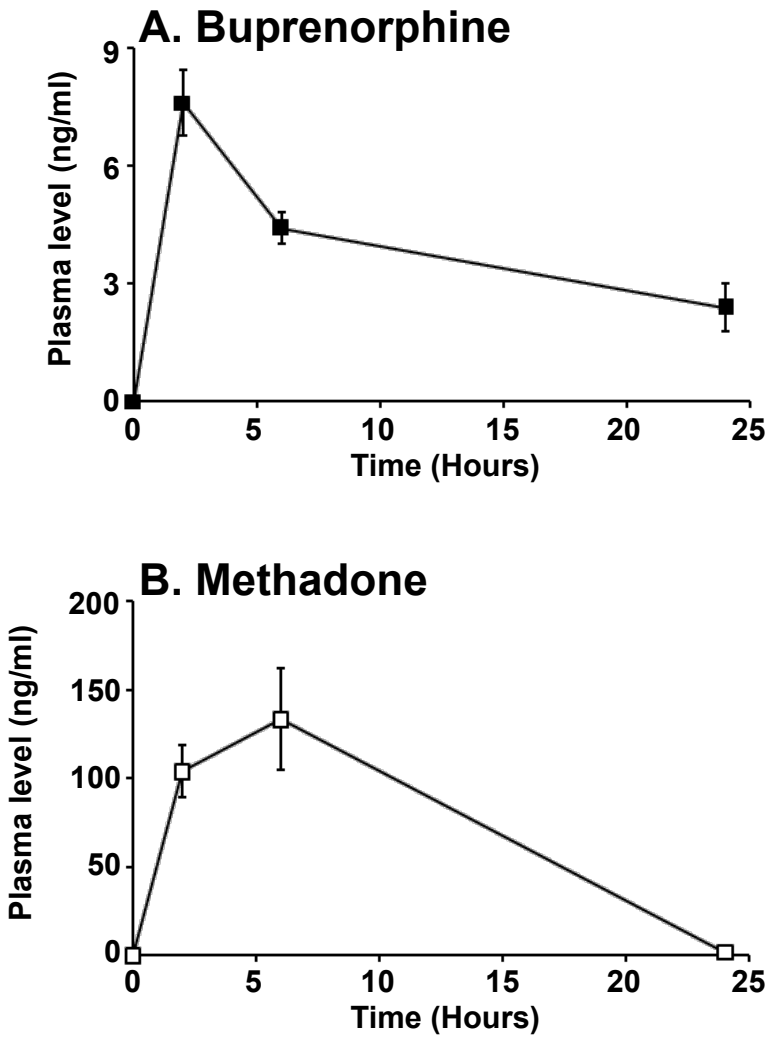

Figure 5 Mice were orally administered 0.2 mg/kg buprenorphine or 50 mg/kg methadone for 6 days. Plasma levels of buprenorphine (**A**) and methadone (**B**) were examined two, six and 24 hours after the last administration. Results are presented as mean ± SEM.